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TITLE: A Novel Approach to Detect Therapeutic Resistance in Breast Cancer

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CONTRACTING ORGANIZATION: Karolinska Institutet
Stockholm, Sweden

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14. ABSTRACT
Purpose/scope: Our aim is to understand whether adjuvant therapy of a first primary breast cancer might predict the prognosis of a metachronous bilateral cancer. Major findings: Our findings support a selection process where adjuvant systemic treatment selectively prevents the occurrence of cancers with a favourable prognosis, allowing those with a more aggressive phenotype to surface clinically. Up-to-date report: We have requested for a no-cost extension of the project due to difficulties with collection of tissue samples.

15. SUBJECT TERMS
No subject terms provided.

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1. Introduction

Globally, increasing breast cancer incidence rates, improved prognosis and growing life expectancy have resulted in increasing number of women at risk of developing a bilateral primary breast cancer. There are an estimated 2.2 million women living in the US who have been diagnosed at some time with breast cancer [1]. Hence, optimal surveillance and clinical management of women who have had one or two primary breast cancers is a challenge. However, there are only limited data on incidence rates of synchronous and metachronous breast cancer [2,3] , results on temporal trends in incidence are conflicting [4] and little is known about the prognostic outlook following treatment of a second primary cancer [5,6]. Our aim is to understand whether adjuvant therapy of a first primary breast cancer might predict the prognosis of a metachronous bilateral cancer.

2. Body

Research accomplishments as outlined in the Statement of Work.

Task 1. Identification of cases and controls of uni- and bilateral breast cancer for the case-control data file, Months 1:

a. Using the regional cancer register in Stockholm, Sweden we will identify women diagnosed with unilateral breast cancer included in randomized clinical trails 1985-1995, n=3,200
b. Within the cohort of 3,200 unilateral breast cancer cases we will identify those diagnosed with bilateral breast cancer within 5 years of primary cancer, n=150.
c. Using the regional cancer register in Stockholm, Sweden to sample women with bilateral breast cancer within 5 years who died and those who did not, n=50+50.
d. Identifying hospital at which medical records and tissue are located using a unique national registration number.

Summary:
Using the regional cancer register in Stockholm, Sweden we identified 17,089 women diagnosed with unilateral breast cancer, among these women, 441 developed metachronous bilateral disease within 5 years of their primary breast cancer during follow-up through 1999. We identified hospitals at which medical records and tissue are located using a unique national registration number.

Task 2. Abstracting information medical records, months 2-4

a. Constructing the abstracting manual.
b. Structural work of organizing a database to store information in using Oracle®
c. Retrieving medical records from hospital.
d. Abstracting information from medical records into database.
e. A database quality control program will instituted to check for errors and inconsistencies in the database.

Summary:
We constructed the abstracting manual, see Appendix 1. We have completed a structural work of organizing a database to store information in using Oracle®. Of the 441 study participants with short latency contralateral breast cancer, 8.6% was shown at investigating of the medical record to not fulfill the inclusion criteria and was thus excluded, this will naturally increase the precision of the study. Of the remaining 402 patients 91% are collected and computerized and 6% have not been possible to find in the archives. We expect to finish the data collecting step during November.

Regarding the tissue collection we have gained permission from the ethical committee to collect tissue from the biobank and recently also gained permission from the biobank committee to have access to the tissue. The cases for tissue analysis have been selected and ordered from the biobank, we expect the tissue to arrive during the autumn. A pathologist and a lab technician have been affiliated/involved in the project to prepare and investigate the tissue.
A database quality control program was developed to check for errors and inconsistencies in the database and for comparison of the information in the medical records and regional cancer register in Stockholm. When comparing information on treatment from medical records and from register we notice that treatment information is (very) much more complete in the medical record than in the register. Only 2% had any of the treatment variables unknown in the dataset with information collected from medical records. Also variables regarding tumour characteristics, like tumour size, and hormonal information, like menopause status, were more complete in the information from medical records.

Task 3. Data analysis of women with bilateral breast cancer. Months 5:

a. The data analysis will be detailed and analysis of the final data set of bilateral breast cancer will be conducted using SAS statistical software package.
b. Entry and exit information to allow for censor will be created.
c. Using register based information as well as medical records, death due to breast cancer will be determined.
d. Exposure information including type of therapy will be assessed and categorized.

Summary:
1: Analysis using data from regional cancer register in Stockholm
We tested the hypothesis if women treated aggressively for their first breast cancer were more likely to die when diagnosed with a short latency metachronous cancer using a data from regional cancer register in Stockholm (Apendix 2, Table 2).
Our results support the interpretation by showing a stage adjusted 2.4-fold higher mortality rate among women who received adjuvant chemotherapy following their first primary breast cancer, while there is no increased mortality following chemotherapy after the second primary cancer. We believe that the findings support a selection process where adjuvant systemic treatment selectively prevents the occurrence of cancers with a favourable prognosis, allowing those with a more aggressive phenotype to surface clinically.

2: Analysis using data collected from medical records
Our cohort for analysis of possible therapeutic resistance in short latency bilateral cancer comprised 17,089 women, among these women, 441 developed metachronous bilateral disease within 5 years of their primary breast cancer during follow-up through 1999. We included only women with TNM stage 1-3 at first diagnosis in order to minimize the risk of misclassified metastatic disease and further to minimize confounding by indication for adjuvant treatment in relation to bilateral breast cancer death. For all these women all medical records were retrieved and information on treatment, tumor characteristics, hormonal information etc were ascertained and computerized. To date 91% of 441 women with metachronous cancers diagnosed within 5 years have been retrieved and these women were included in the analysis.
The occurrence of distant metastasis was used as a measure of prognosis and was ascertained from the Regional Oncological Center, Stockholm as well as the medical records. Incidence rate of distant metastasis was calculated with the accumulated person-time at risk as the denominator. This time started at second diagnosis for bilateral breast cancer and continued until occurrence of distant

metastasis, emigration, death or end of follow-up (December 31, 1999), whichever came first. Information on adjuvant treatment and receptor status was ascertained from the medical records.
Poisson regression was used for modeling of occurrence of distant metastasis. We adjusted for age and calendar period of diagnosis and time since diagnosis. Further adjustment was made for TNM stage, oestrogen receptor status and adjuvant treatment. We present 5-year cause specific mortality and follow-up for distant metastasis. Follow-up was censored at age 80 years because classification of cause of death may be less reliable in older women.
The effect of adjuvant treatment on occurrence of distant metastasis among women diagnosed with metachronous cancer within 5 years of primary cancer is presented in Table 1. Women who had received any form of systemic adjuvant treatment were 3.2 times (95% CI 1.1-9.0) more likely to develop a distant metastasis compared to women who received no adjuvant treatment when we adjusted for follow-up time, age, calendar period, TNM stage, hormone receptor status, and adjuvant treatment of the second cancer. Women who received adjuvant chemotherapy were 4.7 times (95% CI 1.4-16.1) more likely to develop a distant metastasis compared to women who received no adjuvant treatment with similar adjustment.

Table 1

Incidence rate ratio (IRR) of distant metastasis among women who developed metachronous bilateral disease within 5 years of their primary breast cancer in relation to adjuvant treatment of first primary cancer.

Type of treatment	**IRR**	**95%CI**
Any adjuvant treatment	3.2	1.1-9.0
Only Hormonal therapy	1.9	0.6-6.0
Only Chemotherapy	4.7	1.4-16.1
Hormonal therapy and Chemotherapy	3.0	0.7-12.7
No adjuvant tratment	1.0	reference

CI, 95 percent confidence intervals

Task 4. Analysis of tissue, Months 6-7:

a. A quality assurance protocol will be instituted to check the specimen quality, n=50+50.
b. Fluorescence in situ hybridization analysis (QM-FISH) will setup and standardized.
c. Analysis of gene copy number (amplification and deletion) with array technology will be setup and standardized.
d. Molecular analysis of cases who died and controls who did not will be done.

Summary:
Since the collection and preparation of tissue samples is still ongoing, we did not start with analysis of tissue samples. The delay is due to the new regulations in Sweden. Therefore our focus was so-far on the setup and standardization of QM-FISH method. QM-FISH (Quantitative Multi-gene Fluorescence In-Situ Hybridization) developed by Anders Zetterberg's lab will be used to study gene

copy number changes (allelic imbalances) in about 50 selected genes. Starting from the conventional FISH technique, he has developed a quantitative, multi-gene technique which enables us to study gene copy number changes (allelic imbalances) as can be seen in Figure 1. In a larger study conducted by Zetterberg et al., genetic rearrangements were identified that are associated with worse survival in breast cancer patients, even after accounting for tumor grade, ER status, progesterone status, node status and tumor size [7].

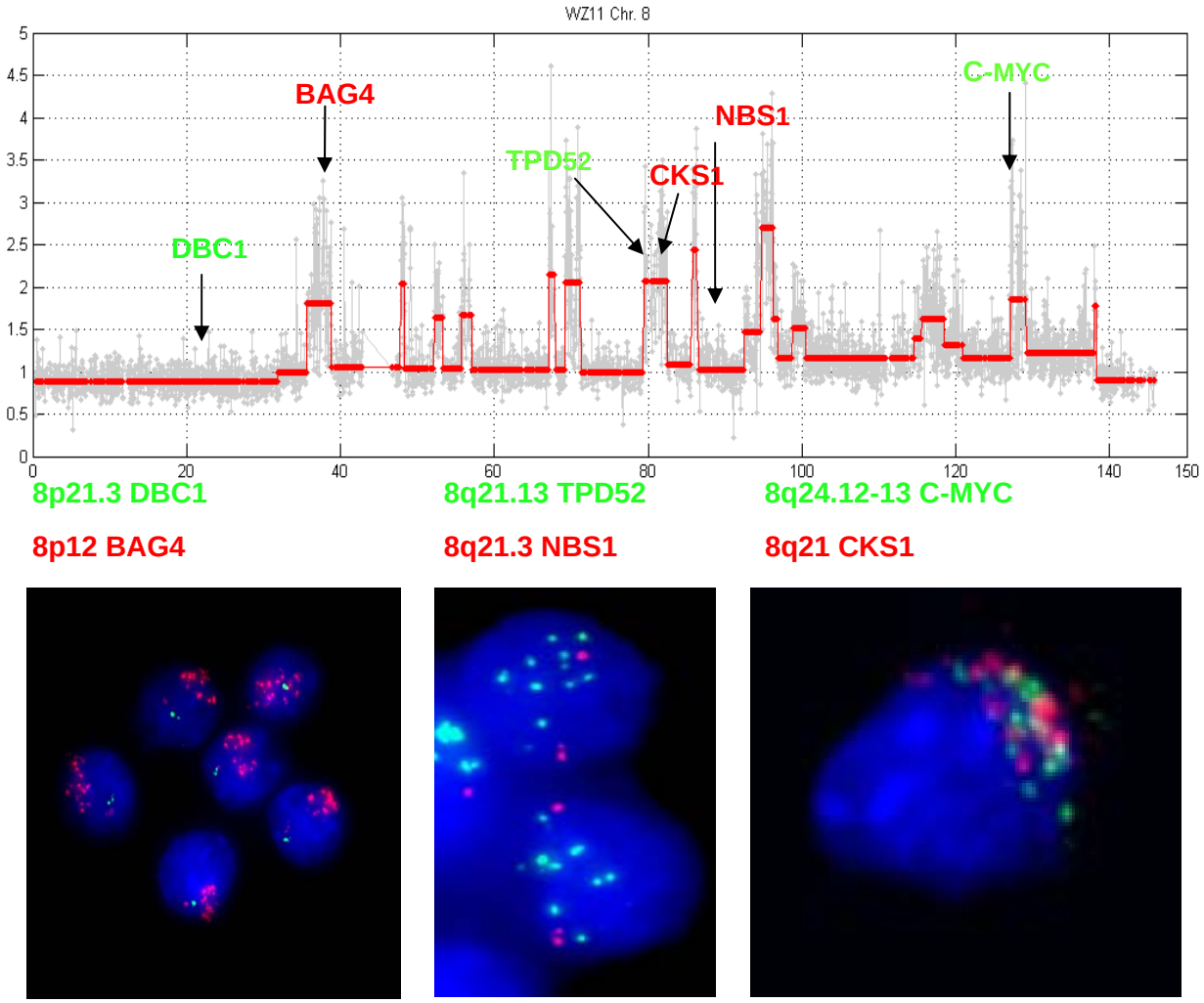

Figure 1. QM-FISH has previously been used to validate amplifications and deletions identified in breast cancers by interphase FISH. Aberrations on chromosome 8, amplifications (*e.g.* BAG4) and deletions (*e.g.* DBC1), are clearly displayed by QM-FISH in the lower left-hand panel. (Best

As a result of methodological development in Anders Zetterberg's lab, several individual genes can now be identified and quantified accurately and reproducibly in each tumor cell nucleus at the same time which makes the technique suitable for large-scale clinical studies. The technique consists of new protocols for hybridization, 3D-fluorescence microscopy with a Z-scanning devise, a CCD-camera for image acquisition and PCs for control functions and image analysis. The digital image analysis algorithms and software that have been developed at Karolinska Institutet consists of an

optimized combination of existing as well as new powerful algorithms. This has resulted in a QM-FISH-technique with very high sensitivity and specificity. Technique has been developed to generate QM-FISH-probes from any region of the genome. With the current methodology, the influence of FISH-artefacts (loss of signals and/or split signals) has been reduced and gene copy number can be accurately quantified in over 90% of the cells in a cell population.

Task 5. Data analysis of 50 bilateral cases and 50 controls. Months 8-9:

a. The data analysis will be detailed and analysis of the final data set will be conducted using SAS statistical software package

Summary:
Since the collection and preparation of tissue samples is still ongoing, we did not start with data analysis of tissue samples.

Task 6. Report and manuscript preparation, Months 10-12:

a. Manuscripts will be prepared.
b. A final report of the findings will be written.

Summary:
One manuscript has been written using our results from register-based studies, see Appendix 2. One more manuscript is in preparation based on data collected from medical records. We are planning the third manuscript on molecular studies of prognosis of bilateral breast cancers with short latency.

3. Key research accomplishments

1) Identification of metachronous bilateral breast cancers and collection of data for 91% of cases.
2) Identification of tissue samples and ongoing preparation of samples for molecular analysis
3) Analysis of data collected from medical records showing that adjuvant treatment selectively prevents the occurrence of cancers with a favourable prognosis, allowing those with a more aggressive phenotype to surface clinically.

4. Reportable outcomes

1) Article: Incidence and prognosis of synchronous and metachronous bilateral breast cancer
Mikael Hartman M.D, Kamila Czene Ph. D., Marie Reilly Ph.D. , Jan Adolfsson M.D., Ph.D. , Jonas Bergh M.D., Ph.D, Hans-Olov Adami M.D., Ph. D. Paul W. Dickman Ph.D., Per Hall M.D., Ph.D.
Journal of clinical Oncology, in press.

2) Dissertation, Mikael Hartman, Thesis: Risk and prognosis of breast cancer among women at high risk of the disease, Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, October 12, 2007

3) Abstract: Incidence and prognosis of synchronous and metachronous bilateral breast cancer, The 29th Annual San Antonio Breast Cancer Symposium, December 14 - 17, 2006 in San Antonio, Texas.

5. Conclusion

Women with bilateral breast cancer have a poor survival. Predictors of a poor outcome include young age at first cancer, a second diagnosis within 5 years and those treated with adjuvant therapy for the first cancer.

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7. Hicks J, Krasnitz A, Lakshmi B, et al: Novel patterns of genome rearrangement and their association with survival in breast cancer. Genome Res 16:1465-79, 2006

7. Appendices

1) Abstract form for data collection from medical records
2) Incidence and prognosis of synchronous and metachronous bilateral breast cancer.
Mikael Hartman M.D, Kamila Czene Ph. D., Marie Reilly Ph.D. , Jan Adolfsson M.D., Ph.D. , Jonas Bergh M.D., Ph.D, Hans-Olov Adami M.D., Ph. D. Paul W. Dickman Ph.D., Per Hall M.D., Ph.D.
Journal of clinical Oncology, in press.

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APPENDIX 1

Uppdaterad: 2007-4-20 /MS

RABBIT

- Bilateral Breast cancer studied from patient Information and Tissue

Abstract form -metachronous

Patient Sequence No. ☐

Registered in the data base ☐

Exkluderad ☐

Date of abstraction: ☐
Code: yyyy-mm-dd

Abstractor: ☐
Code: abstractors initials

First diagnose date: ☐
Code: yyyy-mm-dd

Second diagnose date: ☐
Code: yyyy-mm-dd

Notes:

.....

.....

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Patient information at time for first breast cancer diagnosis

Socio-economical status

Profession

Relevant earlier diseases

Earlier benign breast disease: Code: 1= yes, 2= no, 998= unknown

Diabetes: Code: 1= yes, 2= no, 998= unknown

Thyroid disease: Code: 1= yes, 2= no, 998= unknown

Schizophrenia Code: 1= yes, 2= no, 3=other psychiatric illness, 998= unknown

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Smoking info (at time of first breast cancer diagnosis)

Smoker Code: 1= current, 2= no, 998= unknown

If no: Code: 1= never, 2 = previous smoker, 998= unknown,

Hormone related info

Menarche Code: age or 998= unknown

Pregnancy Code: 1=yes, 2= no, 998= unknown

If yes, give number Code: number, 998=unknown,

Parity Code: 1=yes, 2= no, 998= unknown

If yes, give number Code: number, 998=unknown,

First partus Code: age, 998= unknown,

Breast feeding Code: 1= yes, 2= no, 998= unknown,

Menopause *at time of breast cancer diagnosis* Code: 1= yes, 2= no, 998= unknown

Menopause, age Code: age or 998= unknown,

Oral contraceptive *at time of breast cancer diagnosis* Code: 1= current, 2= no, 998= unknown

If no; Code: 1= never, 2 = previous, 998= unknown,

Hormone replacement Therapy at time of breast cancer diagnosis Code: 1= current, 2= no, 998= unknown

If no; ☐ Code: 1= never, 2 = previous, 998= unknown,

Anthropometric measures

Height: ☐ Code: height in meters or 998 = unknown

Weight: ☐ Code: weight in kilos or 998 = unknown

Build/physique: ☐ Code: 1= overweight, 2= normal, 3=underweight, 998= unknown

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Heredity (at time of first breast cancer diagnosis)

Family history of breast cancer ☐ Code: 1= yes, 2= no, 998= unknown

Family history of ovarian cancer ☐ Code: 1= yes, 2= no, 998= unknown

Relationship ☐ Code: 1= parent, 2= sibling, 3= offspring, 4= more distant, 998=unknown,

Type of cancer ☐ Code: 1= breast, 2= ovary, 3= both,

Relationship ☐ Code: 1= parent, 2= sibling, 3= offspring, 4= more distant,

Type of cancer ☐ Code: 1= breast, 2= ovary, 3= both,

Relationship ☐ Code: 1= parent, 2= sibling, 3= offspring, 4= more distant,

Type of cancer ☐ Code: 1= breast, 2= ovary, 3= both,

Relationship ☐ Code: 1= parent, 2= sibling, 3= offspring, 4= more distant,

Type of cancer ☐ Code: 1= breast, 2= ovary, 3= both,

First Breast cancer

Date of diagnosis ☐ Code: yyyy-mm-dd or 998= unknown

Location ☐ Code: 1= right, 2= left, 3= bilateral, 998= unknown

PAD no. ☐ Code: Actual number, 998=unknown, 777=PAD do not exist

PAD report copied ☐ Code: 1= yes, 2= no,

Mode of detection ☐ Code: 1= palpation by patient, 2= screening mammography, 3= palpation by MD, 4= control mammography, 5=other, 998= unknown

If other; name it ☐

Surgical info - First Breast cancer

Surgery ☐ Code: 1= yes, 2= no, 998= unknown

Surgery, date [ ] Code: yy-mm-dd or 998= unknown,

Surgery, type [ ] Code: 1= partial mastectomy, 2= total mastectomy, 3= biopsy, 998= unknown,

Lymph nodes examined [ ] Code: 1= yes, 2= no, 998= unknown

If yes, positive findings [ ] Code: 1= yes, 2= no, 998= unknown,

Number of positive lymph nodes [ ] out of [ ] examined lymph nodes

Code: actual number, 998= unknown,

Periglandular growth [ ] Code: 1= yes, 2= no, 998= unknown

Tumor characteristics -First breast cancer

Information source [ ] Code: 1= PAD/ tissue analysis, 2= cytology, 998= unknown,

Histology [ ] Code: 1= ductal, 2= lobular, 3= other, 998= unknown

If other (=3), specify [ ]

Multiple tumors [ ] Code: 1= yes, 2= no, 998= unknown

Further comments [ ]

Largest tumor size [ ] Code: size in mm (given in PAD) or 998= unknown

Tumor cell differentiation [ ] Code: 1= high, 2= intermediate, 3= low, 998= unknown

Estrogen receptor status [ ] Code: 1= positive, 2= negative, 3=positive, unclear laterality, 4= negative, unclear laterality 998= unknown

Value: [ ] Code: value [ ] Code: unit

Progesterone receptor status [ ] Code: 1= positive, 2= negative, 3=positive, unclear laterality, 4= negative, unclear laterality 998= unknown

Value: [ ] Code: value [ ] Code: unit

S-phase [ ] % Code: value in percent or 998=unknown

Treatment -First Breast cancer

<u>Radiotherapy (RT)</u> [ ] Code: 1= yes, 2= no, 998= unknown

RT, type [ ] Code: 1= preoperative, 2= postoperative , 3= not surgically treated 998= unknown

RT, start date [ ] Code: yy-mm-dd or 998= unknown,

Chemotherapy (CT) neoadjuvant [ ] Code: 1= yes, 2= no, 998= unknown

CT used [ ] Code: 1= FEC/FAC, 2=Taxotere, 3= CMF, 4= other, 998= unknown,

If other, specify: [ ]

CT, start date [ ] Code: yy-mm-dd, 998=unknown ,

Duration of CT [ ] Code: Actual number of cycles, 888=continuous, 998=unknown,

Further comments [ ]

Adjuvant chemotherapy (CT) [ ] Code: 1= yes, 2= no, 998= unknown

CT used [ ] Code: 1= FEC/FAC, 2= Taxotere, 3= CMF, 4= other, 998= unknown,

If other, specify: [ ]

CT, start date [ ] Code: yy-mm-dd, 998= unknown,

Duration of CT [ ] Code: Actual number of cycles, 888=continuous, 998=unknown,

Further comments [ ]

Adjuvant hormone therapy (HT) [ ] Code: 1= yes, 2= no, 998= unknown

HT used [ ] Code: 1= Tamoxifen only, 2=other, 998= unknown,

If other (=2), specify [ ]

Dose of HT [ ] Code: daily dose (in mg), 998= unknown,

HT, intended time of use [ ] Code: months, 998=unknown,

Recurrent tumors -First breast cancer

New, local or regional ipsilateral tumor [ ] Code: 1= yes, 2= no, 998= unknown,

Type of recurrent tumor [ ] Code: 1=local, 2=regional, 998=unknown, 222=not applicable

Date of diagnosis [ ] Code: yy-mm-dd,

Surgery, recurrent tumor [ ] Code: 1=yes, 2=no, 998=unknown,

PAD no. [ ] Code: Actual number, 998=unknown, 777=PAD do not exist

PAD report copied [ ] Code: 1= yes, 2= no,

RT, recurrent tumor [ ] Code: 1= yes, 2= no, 998= unknown,

CT, recurrent tumor [ ] Code: 1= yes, 2= no, 998= unknown,

CT Used [ ] Code: 1= FEC/FAC, 2= Taxotere, 3= CMF, 4= other, 998= unknown,

If other, specify: [ ]

CT, start datum [ ] Code: yy-mm-dd, 998= unknown, 222=not applicable

Duration of CT [ ] Code: Actual number of cycles, 888=continuous, 998=unknown,

Further comments [ ]

HT, recurrent tumor [ ] Code: 1= yes, 2= no, 998= unknown ,

Second breast cancer

Date of diagnosis [ ] Code: yy-mm-dd or 998= unknown

Diagnosed during treatment [ ] Code: 1= yes, treatment for BCI, 2= no, 3=yes, treatment for recurrences

Laterality [ ] Code: 1= right, 2= left, 3= bilateral, 998= unknown

PAD no. [ ] Code: Actual number, 998=unknown,

PAD report copied [ ] Code: 1= yes, 2= no

Mode of detection [ ] Code: 1= palpation by patient, 2= screening mammography, 3= palpation by MD, 4= control mammography, 5=other, 998= unknown

If other; specify [ ]

Further comments [ ]

Surgical info –second breast cancer

Surgery [ ] Code: 1= yes, 2= no, 998= unknown

Surgery, date [ ] Code: yy-mm-dd or 998= unknown,

Surgery, type [ ] Code: 1= partial mastectomy, 2= total mastectomy, 3= biopsy, 998= unknown,

Lymph nodes examined [ ] Code: 1= yes, 2= no, 998= unknown

If yes, positive findings [ ] Code: 1= yes, 2= no, 998= unknown,

Number of positive lymph nodes [ ] out of [ ] examined lymph nodes

Code: actual number, 998= unknown,

Periglandular growth [ ] Code: 1= yes, 2= no, 998= unknown

Tumor characteristics –second breast cancer

Information source [ ] Code: 1= PAD/ tissue analysis, 2= cytology, 998= unknown,

Histology [ ] Code: 1= ductal, 2= lobular, 3= other , 998= unknown

If other (=3), specify [ ]

Multiple tumors [ ] Code: 1= yes, 2= no, 998= unknown

Further comments [ ]

Largest tumor size [ ] Code: size in mm (given in PAD) or 998= unknown

Tumor cell differentiation [ ] Code: 1= high, 2= intermediate, 3= low, 998= unknown

Estrogen receptor status [ ] Code: 1= positive, 2= negative, 3=positive, unclear laterality, 4 =negative, unclear laterality 998= unknown

Value: [ ] Code: value [ ] Code: unit

Progesterone receptor status [ ] Code: 1= positive, 2= negative, 3=positive, unclear laterality, 4 = negative, unclear laterality 998= unknown

Value: [ ] Code: value [ ] Code: unit

S-phase [ ] % Code: value in percent or 998=unknown

Treatment -second breast cancer

<u>Radiotherapy (RT)</u> [ ] Code: 1= yes, 2= no, 998= unknown

RT, type [ ] Code: 1= preoperative, 2= postoperative , 3= not surgically treated 998= unknown

RT, start date [ ] Code: yy-mm-dd or 998= unknown,

<u>Chemotherapy neoadjuvant</u> [ ] Code: 1= yes, 2= no, 998= unknown

CT used [ ] Code: 1= FEC/FAC, 2=Taxotere, 3= CMF, 4= other, 998= unknown,

If other, specify: [ ]

CT, start date [ ] Code: yy-mm-dd or 998= unknown,

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Further comments [ ]

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Recurrent tumors -Second Breast cancer

New, local or regional ipsilateral tumor [ ] Code: 1= yes, 2= no, 998= unknown,

Type of recurrent tumor [ ] Code: 1=local, 2=regional, 998=unknown, 222=not applicable

Date of diagnosis [ ] Code: yy-mm-dd,

Surgery, recurrent tumor [ ] Code: 1=yes, 2=no, 998=unknown,

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PAD report copied [ ] Code: 1= yes, 2= no,

RT, recurrent tumor [ ] Code: 1= yes, 2= no, 998= unknown,

CT, recurrent tumor [ ] Code: 1= yes, 2= no, 998= unknown,

CT Used [ ] Code: 1= FEC/FAC, 2= Taxotere, 3= CMF, 4= other, 998= unknown,

If other, specify: [ ]

CT, start datum [ ] Code: yy-mm-dd, 998= unknown, 222=not applicable

Duration of CT [ ] Code: Actual number of cycles, 888=continuous, 998=unknown,

Further comments [ ]

HT, recurrent tumor [ ] Code: 1= yes, 2= no, 998= unknown,

Distant metastasis after 2nd BC diagnosis [ ] Code: 1= yes, 2= no, 998= unknown

Date of met. [ ] Code: yy-mm-dd, 998= unknown,

Diagnosed during treatment for 2nd BC [ ] Code: 1= yes, 2= no, 998= unknown

Localization of first distant metastasis [ ] Code: 1 = lung, 2 = liver, 3 = skeleton, 4 = brain, 5 = other, 998= unknown,

If other, specify: [ ]

Last date of follow-up [ ]

Additional PADs

PAD no. [ ] Code: Actual number, 998=unknown,

PAD report copied [ ] Code: 1= yes, 2= no,

Type of tissue, specify [ ]

Date on PAD report: [ ] Code: actual date or 998=unknown

PAD no. [ ] Code: Actual number, 998=unknown,

PAD report copied [ ] Code: 1= yes, 2= no,

Type of tissue, specify [ ]

Date on PAD report: [ ] Code: actual date or 998=unknown

PAD no. [ ] Code: Actual number, 998=unknown,

PAD report copied [ ] Code: 1= yes, 2= no,

Type of tissue, specify [ ]

Date on PAD report: [ ] Code: actual date or 998=unknown

PAD no. [ ] Code: Actual number, 998=unknown,

PAD report copied [ ] Code: 1= yes, 2= no,

Type of tissue, specify [ ]

Date on PAD report: [ ] Code: actual date or 998=unknown

Hormone related info –second breast cancer

Menopause *at time of breast cancer diagnosis*	☐	Code: 1= yes, 2= no, 998= unknown
Menopause, age	☐	Code: age or 998= unknown,
HRT *at time of breast cancer diagnosis*	☐	Code: 1= current, 2= no, 998= unknown
If no;	☐	Code: 1= never, 2 = previous, 998= unknown,

Mammography info –*closest to first breast cancer diagnosis*

Mammography, copy of	-pictures	☐	Code: 1= yes, 2= no original available
	-density scaling	☐	Code: 1= yes, 2= no original available
Date of mammography:		☐	Code yy-mm-dd (as close to first diagnosis as possible), 998=unknown
Second opinion of mammography pictures:		☐	Code: 1= yes, 2= no, 998= unknown
	Density scaling	☐	Code:

Comments

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APPENDIX 2

Incidence and prognosis of synchronous and metachronous bilateral breast cancer

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Word count: 3,021

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ABSTRACT

Purpose: Because the incidence of breast cancer is increasing and prognosis is improving, a growing number of women are at risk of developing bilateral disease. Little is known, however, about incidence trends and prognostic features of bilateral breast cancer.

Patients and Methods: Among 123,757 women with a primary breast cancer diagnosed in Sweden from 1970 to 2000, altogether 6,550 developed bilateral breast cancer. We separated synchronous (diagnosed within three months after a first breast cancer) and metachronous bilateral cancer and analyzed incidence and mortality rates of breast cancer using Poisson regression models.

Results: The incidence of synchronous breast cancer increased by age and by 40% during the 1970s, whilst the incidence of metachronous cancer decreased by age and by about 30% since the early 1980s most likely due to increasing use of adjuvant therapy. Women who developed bilateral cancer within 5 years and before age 50 were 3.9 times (95% CI 3.5-4.5) more likely to die from breast cancer than women with unilateral cancer. Women with a bilateral cancer diagnosed more than 10 years after the first cancer had a prognosis similar to that of a unilateral breast cancer. Adjuvant chemotherapy of primary cancer is a predictor of poor survival after diagnosis of early metachronous cancers.

Conclusion: We found profound differences in the incidence trends and prognostic outlook between synchronous and metachronous bilateral breast cancer diagnosed at different ages. Adjuvant chemotherapy therapy has a dual effect on metachronous cancer; it reduces the risk, while at the same time it seems to worsen the prognosis.

Word count: 248

INTRODUCTION

Globally, increasing breast cancer incidence rates, improved prognosis and growing life expectancy have resulted in increasing number of women at risk of developing a bilateral primary breast cancer. There are an estimated 2.2 million women living in the US who have been diagnosed at some time with breast cancer [1]. Hence, optimal surveillance and clinical management of women who have had one or two primary breast cancers is a challenge. However, there are only limited data on incidence rates of synchronous and metachronous breast cancer [2,3] , results on temporal trends in incidence are conflicting [4] and little is known about the prognostic outlook following treatment of a second primary cancer [5,6].

We analyzed a large nation-wide cohort of breast cancer patients in Sweden. We achieved complete follow-up with regard to incidence and survival during 1970 through 2000, a period when mammographic screening and adjuvant systemic treatment became broadly implemented. We also analyzed a separate cohort with detailed treatment information in order to understand better whether adjuvant chemotherapy of a first primary breast cancer might predict the prognosis of a metachronous bilateral cancer.

METHODS

Study Cohort

The study cohort was obtained from the nation-wide Swedish Cancer Register, established in 1958 and estimated to be at least 98 percent complete [7]. For each notified cancer, the register includes the individually unique national registration number, the ICD-code, and the date of diagnosis but no information on stage of disease or treatment. Using the national registration number, the Cancer Register

can be linked to the nation-wide Cause of Death Register and the Total Population Register. These linkages allow complete follow-up with regard to vital status, date and causes of death, as well as dates of immigration/emigration.

Because laterality of breast cancer was not recorded prior to 1970, we restricted the study cohort to the 138,372 women with a first primary invasive breast cancer diagnosed in the period 1970-2000. We excluded 8,123 women for whom the history of breast cancer was uncertain because they had immigrated to Sweden and 6,492 women with a primary malignant tumor other than in the breast prior to the first breast cancer. Hence, our cohort for final analysis comprised a total of 123,757 women.

Validation cohort

To take treatment and stage into consideration we selected a validation cohort. Since 1976 all new primary breast cancers in the Stockholm-Gotland Health Care Region were reported to a central breast cancer register at the Regional Oncological Center, Stockholm (http://www.sll.se/oc). The register holds information on stage, estrogen and progesterone receptor status, and adjuvant treatment. We identified 19,446 women with a first primary cancer. After excluding 2,357 women with a primary malignant tumor other than breast prior to the first breast cancer, our validation cohort for analysis comprised 17,089 women. Among these women, 952 developed contralateral breast cancer during follow-up through 1999. We included only women with TNM stage 1-3 at first diagnosis in order to minimize the risk of misclassified metastatic disease and further to minimize confounding by indication for adjuvant treatment in relation to bilateral breast cancer death. Hence, our validation cohort for final analysis comprised a total of 900 women with contralateral breast cancer and 16 320 women with unilateral cancer.

Statistical analysis

Unilateral breast cancer incidence rates were calculated using Swedish female population counts as denominators. Bilateral cancers diagnosed within three months of the first primary were categorized as synchronous, the remainder as metachronous. Because synchronous bilateral breast cancer was regarded as a simultaneous clinical event, the incidence rate was calculated as for unilateral breast cancer. The incidence rate of metachronous breast cancer was calculated using as the denominator the accumulated person-years at risk among women with unilateral breast cancer. The person-time at risk started 3 months after the date of first diagnosis and continued until diagnosis of bilateral breast cancer or of any other malignant disease, emigration, death, or end of follow-up (December 31, 2000), whichever came first.

Deaths due to breast cancer were ascertained from the Cause of Death Registry with high reported accuracy [8]. The mortality rate was calculated with the accumulated person-time at risk as the denominator. This time started at first diagnosis for unilateral and at second diagnosis for all bilateral breast cancer and continued until diagnosis of bilateral cancer (for unilateral cancer), emigration, death, or end of follow-up (December 31, 2000), whichever came first. Any in situ breast cancer either prior to or following the first primary cancer was ignored.

We used the Nelson-Aalen method to estimate cause specific cumulative mortality. Poisson regression was used for modeling of both bilateral breast cancer incidence and survival. We adjusted for age and calendar period of diagnosis in the incidence analysis with further adjustment for time since diagnosis in the survival analysis. Within the validation cohort further adjustment was made for TNM stage, oestrogen receptor status and adjuvant treatment. We present 5-year cause specific

mortality, with the exception of Nelson-Aalen cause specific cumulative mortality where we present complete follow-up. We censored follow-up at age 80 years because classification of cause of death may be less reliable in older women.
All data preparation and analysis was done using the SAS Statistical package, version 8.2 [9].

RESULTS

Incidence of bilateral breast cancer

In the cohort of 123,757 women with a first breast cancer diagnosed between 1970 and 2000, altogether 6,550 women developed synchronous (n=1893) or metachronous (n=4657) bilateral breast cancer during follow-up through 2000. Overall, approximately 1.6 synchronous cancers occurred per 10^5 person-years at risk. The incidence of synchronous cancer increased from 1970 until the mid 80's and remained almost constant thereafter (Figure 1). The incidence rate of metachronous cancer decreased by almost one third over the study period from $640/10^5$ in 1970 to $440/10^5$ in 2000. This overall decreasing trend was similar for metachronous cancers diagnosed within 5 years of the first primary breast cancer. In a multivariate Poisson regression model of bilateral breast cancer in relation to calendar period adjusted for age, we observe the same increasing trend of synchronous cancer as seen in Figure 1 (p for trend <0.001; data not shown). The multivariate analyses of metachronous bilateral cancer limited to the first 5 years of follow-up revealed a similar and significant decreasing trend during the study period as seen in Figure 1 (p for trend <0.001).

Survival of bilateral breast cancer

Women with synchronous bilateral breast cancer had a higher mortality from breast cancer than women with unilateral disease ($p < 0.001$; Figure 2A); after 10 years of follow-up, the cumulative breast cancer specific mortality was 45 percent (95% CI 41.4-48.0) and 33 percent (95% CI 32.8-33.5), respectively. Among women with metachronous breast cancer, the lowest mortality from breast cancer was seen for those with the longest time interval between the first and the second cancer (Figure 2B). After 10 years of follow-up, the cumulative breast cancer specific mortality was 56 percent (95% CI 53.0-58.5) among women with bilateral cancer diagnosed within 5 years and 34 percent (95% CI 28.6-39.8) among those diagnosed with bilateral cancer more than 10 years following their first primary.

The 5-year breast cancer specific mortality rate was only modestly related to age at diagnosis among women with unilateral disease (Figure 3A). Following synchronous bilateral breast cancer, mortality decreased from 136 per 10^3 person-years at age <40 years to 73 per 10^3 person-years at age 70-79 years at diagnosis. The modifying effect of age was even more pronounced for metachronous bilateral breast cancer with a more than 3-fold gradient in mortality between women aged <40 years at diagnosis (178 per 10^3 person-years) and those aged 70-79 years at diagnosis (55 per 10^3 person-year).

The 5-year cause-specific mortality rate of synchronous cancer improved continuously during the study period from 124 per 10^3 person-years in 1970-74 to 66 per 10^3 person-years in 1995-2000 (Figure 3B). Similarly, the 5-year cause-specific mortality rate of metachronous breast cancer improved during follow-up from 143 per 10^3 person-years to 68 per 10^3 person-years. This trend was less obvious for

metachronous breast cancer diagnosed less than 5 years since unilateral breast cancer.

We used Poisson regression to estimate how mortality following bilateral breast cancer is affected by age at diagnosis of the first cancer and time interval to diagnosis of second breast cancer (Table 1). Compared to women with unilateral disease, those with synchronous bilateral cancer had a 40 percent higher mortality rate if they were older than 50 years, but a 120 percent higher mortality rate if they were 50 years or younger. Compared to women aged ≤50 years at unilateral breast cancer diagnosis, those who developed a metachronous cancer before 50 years of age within 5 years of their first breast cancer had an almost four-fold higher breast cancer mortality rate. This difference in prognosis between uni- and bilateral disease was reduced in older women and with longer time interval between the two cancers. Women with bilateral metachronous cancers diagnosed more than 10 years after initial diagnosis had a 5-year breast cancer mortality not significantly different to that of women of the same age with a unilateral breast cancer.

In Table 1 we also present results for the validation cohort with further adjustment for TNM stage, estrogen receptor status (negative<0.05 fmol/µg DNA) and adjuvant treatment of primary cancer (for unilateral cancer) and second primary cancer (for bilateral cancer). Women with synchronous cancer had a 60 percent higher mortality rate compared to women with unilateral cancer. Women with metachronous cancer less than 5 years since primary had a more than 4-fold higher mortality rate as compared to women with unilateral cancer. Women with bilateral metachronous cancers diagnosed more than 10 years after initial diagnosis had a 5-year breast cancer mortality not significantly different to that of women of the same age with a unilateral breast cancer.

We next analysed type of bilateral disease (synchronous vs. metachronous) and calendar time – stratified by age at first diagnosis – as determinants of survival (Figure 4A). Women aged ≤50 years diagnosed with synchronous breast cancer in 1970-74 were approximately two times more likely to die from breast cancer than women of the same age with unilateral breast cancer, a difference that varied only modestly over calendar period. In contrast, the excess death rate among women aged ≤50 with metachronous disease increased from less than 3-fold to about 5-fold during the study period. For women >50 years of age at diagnosis of breast cancer, the mortality rate ratio increased steadily from 1.2 to 1.7 (Figure 4B).

We finally analyzed the validation cohort to assess the effect of adjuvant treatment on breast cancer mortality among women diagnosed with metachronous cancer within 5 years of primary cancer (Table 2). Women who had received adjuvant chemotherapy after their first diagnosis were at 2.4-fold higher risk of breast cancer death compared with women who had received no such treatment when we adjusted for follow-up time, age, calendar period, TNM stage, hormone receptor status, and adjuvant treatment of the second cancer. Analysis of the mortality in women with TNM stage 1-2 at primary diagnosis revealed a similar significant 2-fold excess mortality rate following adjuvant chemotherapy of primary cancer. For comparison, we calculated the mortality in women with bilateral disease depending on having received adjuvant chemotherapy for the second primary cancer (Table 2). We observe no excess mortality in women subjected to adjuvant chemotherapy following the second primary cancer. In addition, we observed that women diagnosed with a first primary TNM stage 2 and 3 cancer were at a 2-fold (MRR 1.9; 95%CI 1.0-3.9) and 5-fold (MRR 5.4; 95%CI 2.4-12.1) increased risk to die of the disease respectively compared to women with stage 1 first primary cancer. In comparison,

women diagnosed with a second primary TNM stage 2 and 3 were at a 3-fold (MRR 2.6; 95%CI 1.4-5.1) and 7-fold (MRR 7.2; 95%CI 3.3-15.8) increased risk to die of the disease respectively compared to women with stage 1 first primary cancers.

DISCUSSION

We analyzed the occurrence pattern and prognosis of bilateral breast cancer and found marked differences between synchronous and metachronous cancer during the 30 year period of our study. The incidence pattern of synchronous cancer is similar to that of unilateral disease though without any notable trends in recent decades. Metachronous disease, on the other hand, was much more common in younger patients and incidence rates declined steadily from around 1980 most likely due to the expanding use of adjuvant systemic therapy[10]. Striking features of the survival analyses included the much higher excess mortality following metachronous than synchronous disease, and among younger than older women. When women with metachronous disease were compared with those with unilateral disease the excess mortality increased markedly over calendar-time and was remarkably influenced by time since first breast cancer. The excess mortality of metachronous disease seems to be in part due to treatment of the primary cancer.

Strengths of our study include the large size, the population-based prospective design, the possibility to define laterality, and the completeness of follow-up. Misclassification of metastatic disease as a second primary breast cancer is generally considered a smaller problem and could not explain the pattern of these findings. Indeed, there is no evidence that such misclassification, if it exists, would change over calendar time or differ by age [11]. Admittedly, the prognosis of bilateral breast cancer is difficult to assess; because it is conditional on age and the woman surviving

her first malignancy and deaths cannot be unequivocally attributed to first or second cancer.

The gradual increase in the incidence of synchronous disease during the 1970s coincides with the introduction of routine and bilateral mammography as part of the diagnostic workup in women with unilateral cancer [12]. Such workup may entail that some preclinical bilateral cancers becomes detected early and classified as synchronous disease [13] – perhaps in an earlier and more favourable stage [14] – rather than diagnosed later as metachronous disease. A recent study employing MRI of the opposite breast has demonstrated how intensive clinical workup can increase detection of small tumors [15]. The overall incidence rate of metachronous bilateral cancer in our study is also compatible with previous reports (Figure 1) [3,4,16-18]. During the period of our study, adjuvant systemic therapy, mainly tamoxifen and chemotherapy, became clinical practice. Because such treatment reduces both the incidence of local recurrences, bilateral cancer and distant metastasis [10,19-21] it likely explains the substantial reduction in the incidence of metachronous bilateral disease over calendar time. Similar trends have been found in the US [22] but not in Canada [4].

Women diagnosed with unilateral cancer early in life and bilateral cancer within 5 years had a four times higher mortality rate than women with unilateral breast cancer after adjustment for age at diagnosis and calendar period (Table 1). The pattern persisted but the effect of time since first cancer was weaker in older age groups. In contrast, we observed that women with metachronous cancers diagnosed more than 10 years following initial diagnosis had a prognosis similar to that of a woman with unilateral cancer. This mortality pattern for women with bilateral cancer became even stronger after adjustment of stage and adjuvant treatment in the validation cohort. It is indeed notable that women with metachronous cancer

diagnosed within 5 years following unilateral cancer do worse than women with synchronous bilateral cancer. Our findings are supported by several previous studies[6,23], although there are studies detecting no increased mortality for women with bilateral disease[5,24]. These differences may be attributed to variations in sample size, age and follow-up distributions as well as variation in treatment regimes.

We also found that compared to women with unilateral disease, the prognostic outlook among women with metachronous disease deteriorated over time concomitantly with the decreasing incidence (Figure 4). This novel finding suggests that adjuvant systemic treatment selectively prevents the occurrence of cancers with a favourable prognosis, allowing those with a more aggressive phenotype to surface clinically. Adjuvant chemotherapy is given more often to premenopausal women whilst antiestrogens have been the primary choice among older women. Thus, the much stronger increase in mortality over time among women with bilateral cancer before 50 compared to women with unilateral disease suggests that chemotherapy exerts a stronger selection pressure than adjuvant endocrine treatment. Results from our validation cohort supported this interpretation by showing a stage adjusted 2.4-fold higher mortality rate among women who received adjuvant chemotherapy following their first primary breast cancer (Table 2).

One explanation for this finding would be that chemotherapy is given to women with more aggressive tumors and hence poorer survival. On the other hand, excess mortality following chemotherapy of primary cancer was also observed for women with TNM stage 1-2 cancer, while we observed no increased mortality among women treated with adjuvant chemotherapy following the second primary cancer. We believe that the findings supports a selection process of more malignant second primary

cancers, since the bias by indication for adjuvant chemotherapy should be similar for the choice of treatment of primary and second primary cancer. The result of the validation cohort thus suggests that such bias, if any, to be minor.

Our findings may be relevant both for clinical management of women with breast cancer. It is not surprising that women diagnosed with two simultaneous cancers have an increased mortality as compared to women with one cancer. What is puzzling is the very strong age dependency of this effect. Perhaps even more challenging to explain is the remarkable excess mortality among women who develop short latency metachronous disease at young ages. There is no obvious reason why there should be a difference in biological behaviour between the first and second tumor because the breast tissue is uniformly influenced by the same genetic and environmental factors. Hence, we have to invoke either changes in tumor – host relationships following a first cancer or progression to a therapy resistant phenotype following treatment of the first primary breast cancer.

We found profound differences in the incidence trends and prognostic outlook between synchronous and metachronous bilateral breast cancer diagnosed at different ages. Adjuvant chemotherapy therapy has a dual effect on metachronous cancer; it reduces the risk, while it at the same time seems to worsen the prognosis. Finally, further research into the complex behaviour of bilateral breast cancer may provide important new insight, biologic and clinical.

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Author Contributions: Dr Hartman had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Hall, Hartman, Czene, Reilly, Dickman.

Acquisition of data: Hall, Hartman, Adolfsson.

Analysis and interpretation of data: Hartman, Czene, Dickman,, Bergh, Hall, Reilly, Adami.

Drafting of manuscript: Hartman, Czene, Hall.

Critical revision of the manuscript for important intellectual content: Hartman, Czene, Dickman, Hall, Reilly, Adolfsson, Bergh, Adami.

Statistical analysis: Hartman, Czene, Dickman

Obtained funding: Hartman, Czene, Hall, Reilly

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Figure legends

Figure 1. Temporal trends in incidence rates of unilateral, synchronous and metachronous bilateral breast cancer, in Sweden 1970-2000. 95 % confidence intervals are presented for all studied groups of breast cancer except of metachronous cancer <5 years since primary tumor.

Figure 2. Nelson-Aalen estimates of breast cancer specific mortality following unilateral, synchronous bilateral and metachronous bilateral breast cancer, stratified by time since diagnosis of unilateral breast cancer with follow-up to age 80 years

Figure 3. Breast cancer specific mortality within 5 years following diagnosis of unilateral, synchronous bilateral, and metachronous bilateral breast cancer by age and period of diagnosis in Sweden 1970-2000 with follow-up to age 80 years.

Figure 4. Poisson regression derived mortality rate ratios (MRR) from a Poisson model estimating risk of breast cancer specific mortality following unilateral, synchronous bilateral and metachronous bilateral breast cancer within 5 years of primary cancer by type of breast cancer, calendar period and age at diagnosis of respective type of cancer with follow-up to age 80 years. Reference- unilateral 5-year cause specific mortality rate.

Table 1. Mortality rate ratios (MRR) and 95 percent confidence intervals (CI) from a Poisson model of 5-year cause specific mortality of bilateral breast cancer as compared to unilateral breast cancer using the predictors: age at specified diagnosis and time since unilateral breast cancer diagnosis[*]. In a validation analysis a subcohort of women with TNM stage 1-3 primary cancers from the Stockholm-Gotland Health Care Region was used.

Age at diagnosis (years)	Type of cancer	Time since diagnosis of 1st primary cancer (years)	Deaths	MRR (95 % CI) [*†]
<50	Unilateral[**]		4 739	1.0 ref.
	Synchronous	<0.25	77	2.2 (1.8-2.8)
	Metachronous	0.25-4	263	3.9 (3.5-4.5)
		5-9	52	2.4 (1.8-3.2)
		10-29	11	1.4 (0.8-2.6)
50-79	Unilateral[**]		14 200	1.0 ref.
	Synchronous	<0.25	278	1.4 (1.2-1.6)
	Metachronous	0.25-4	457	1.9 (1.8-2.1)
		5-9	189	1.5 (1.3-1.7)
		10-29	103	1.1 (0.9-1.4)
All ages	Unilateral		18 939	1.0 ref.
	Synchronous	<0.25	355	1.5 (1.4-1.7)
	Metachronous	0.25-4	720	2.4 (2.2-2.6)
		5-9	241	1.6 (1.4-1.9)
		10-29	114	1.1 (0.9-1.4)
Validation cohort[†]				
All ages	Unilateral		1713	1.0 ref.
	Synchronous	<0.25	46	1.7 (1.2-2.2)
	Metachronous	0.25-4	98	4.2 (3.4-5.3)
		5-9	27	2.8 (1.9-4.1)
		10-29	9	1.0 (0.5-2.0)

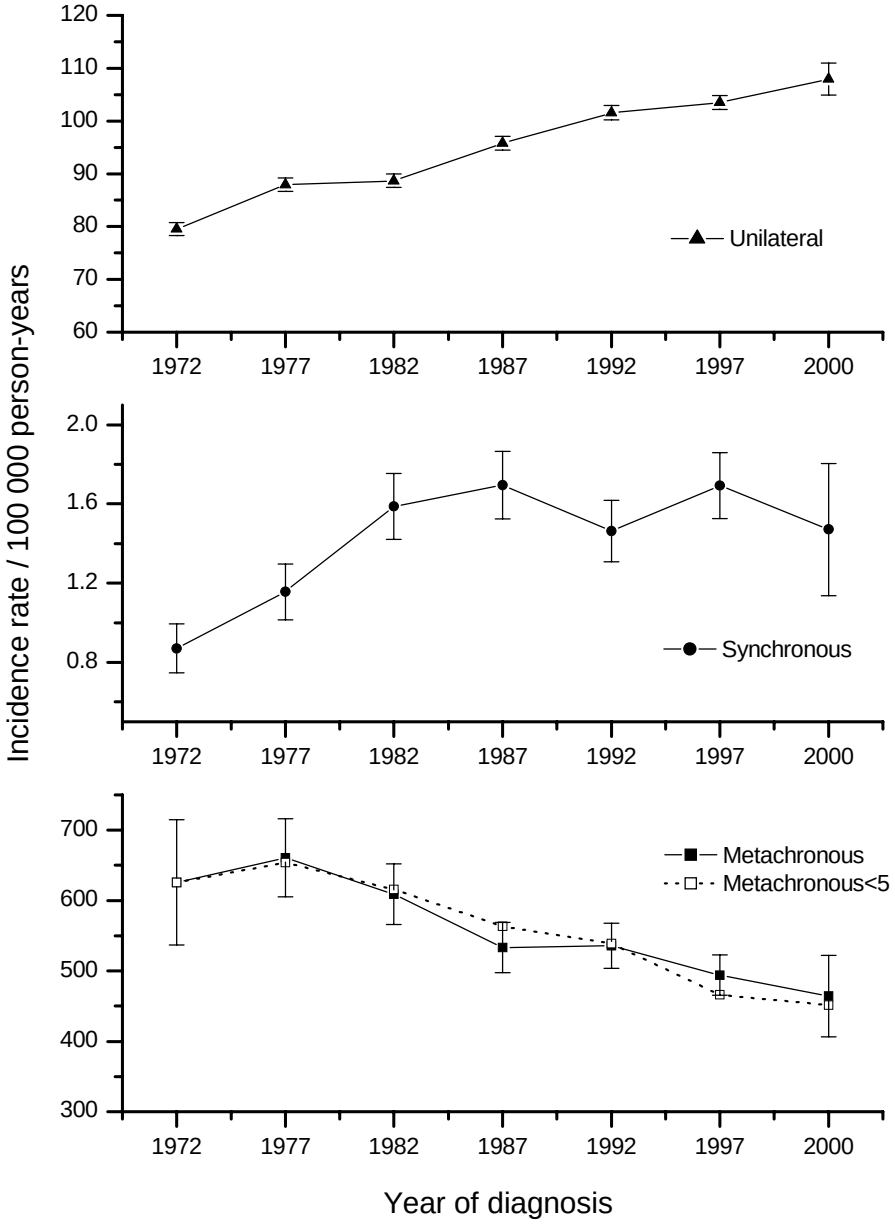
[*]Adjusted for survival time, age and calendar period of diagnosis. [**] Reference: unilateral breast cancer diagnosed at that age. [†] The validation cohort was adjusted for time since diagnosis, age at and calendar period of diagnosis, TNM stage, adjuvant treatment, oestrogen receptor status of primary cancer (for unilateral cancer) and second primary cancer (for bilateral cancer).

Table 2. Mortality rate ratios (MRR) and 95 percent confidence intervals (CI) – obtained from a Poisson model - of 5-year cause specific mortality among women who developed metachronous bilateral disease within 5 years of their primary breast cancer in relation to adjuvant treatment of primary and second primary cancer. Data from the Stockholm-Gotland Health Care Region.

	Number of women	Type of treatment[†]	Number of deaths	MRR (95% CI)
Therapy of 1st cancer[*]				
TNM stage 1-3[α]	171	No chemotherapy	50	1.0 ref
	47	Chemotherapy	27	2.4 (1.3-4.4)
TNM stage 1-2[α]	150	No chemotherapy	34	1.0 ref
	37	Chemotherapy	19	2.2 (1.1-4.6)
Therapy of 2nd cancer[**]				
TNM stage 1-3[α]	130	No chemotherapy	32	1.0 ref
	50	Chemotherapy	10	1.2 (0.5-2.9)
TNM stage 1-2[α]	119	No chemotherapy	22	1.0 ref
	45	Chemotherapy	9	1.1 (0.4-2.8)

[α] TNM stage at primary diagnosis. [†] Chemotherapy is defined as exposed to systemic adjuvant chemotherapy with/without hormonal therapy and radiotherapy. No chemotherapy is defined as never exposed to systemic adjuvant chemotherapy. [*]Adjusted for time since diagnosis, age and calendar period of diagnosis, TNM stage of first and second cancer, oestrogen receptor status of first and second cancer and adjuvant treatment of 2nd cancer [**]Adjusted for time since diagnosis, age and calendar period of diagnosis, TNM stage of first and second cancer, oestrogen receptor status of first and second cancer and adjuvant treatment of 1st cancer.

Figure 1.


Incidence rate / 100 000 person-years
Year of diagnosis
Unilateral
Synchronous
Metachronous
Metachronous<5
120
110
100
90
80
70
60
2.0
1.6
1.2
0.8
700
600
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300
1972
1977
1982
1987
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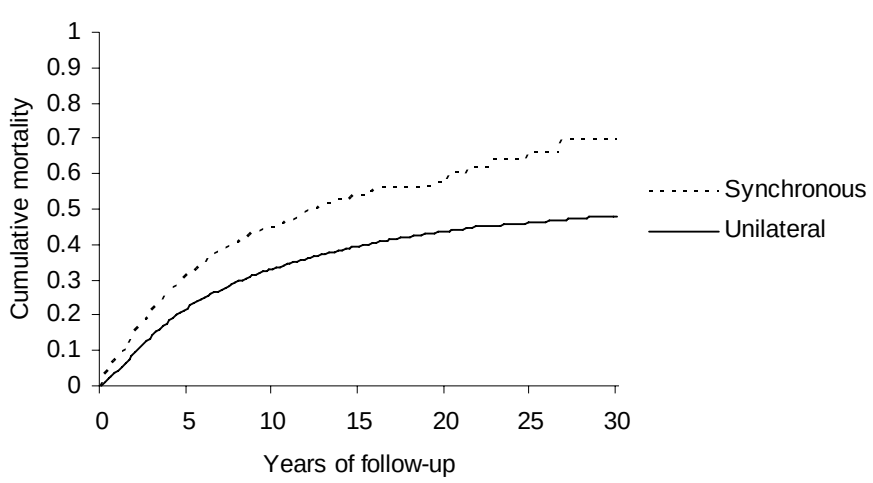
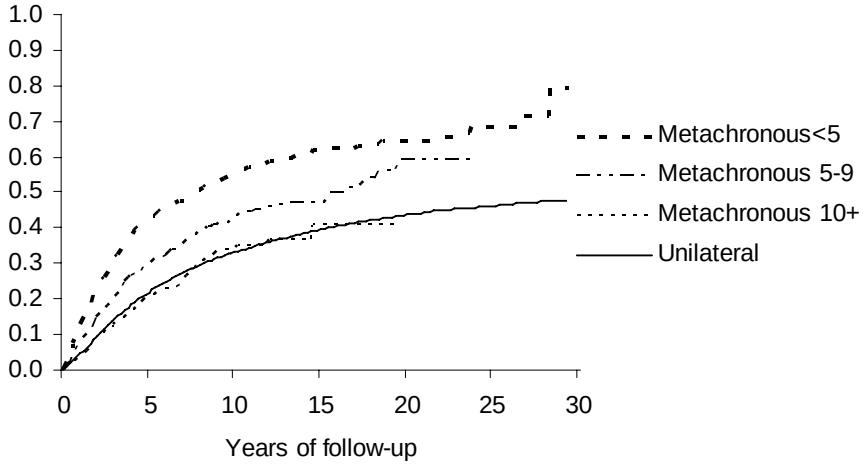
2000

Figure 2.

Panel A



Panel B

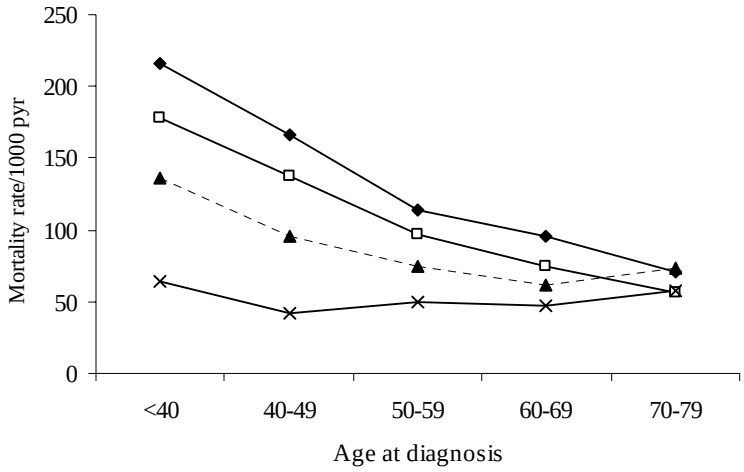


Numbers at risk

Years of follow-up	0	5	10	15	20	30
Unilateral	104 625	42 462	20 135	9 227	3 778	91
Synchronous	1 481	477	199	74	27	1
Metachronous <5 yr	1 996	592	243	102	35	0
Metachronous 5-9 yr	1 036	299	111	42	7	0
Metachronous 10+ yr	798	195	45	6	0	0

Figure 3.

Panel A



Panel B.

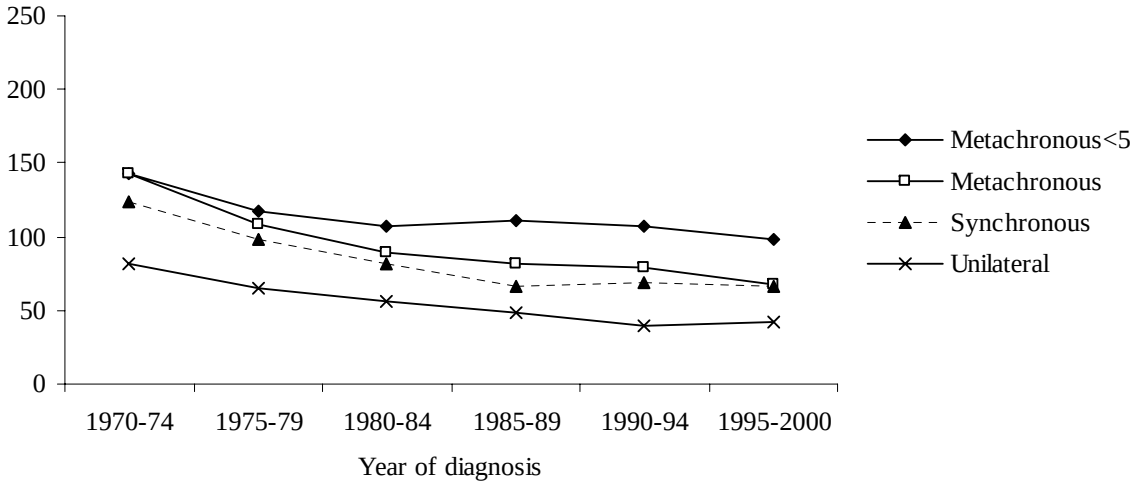
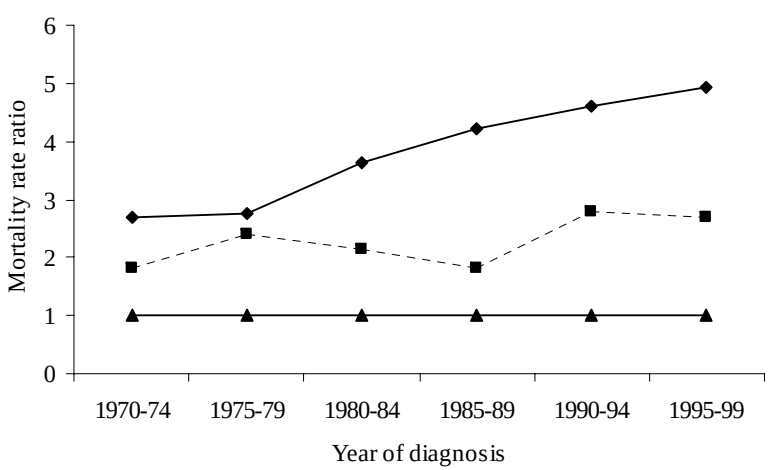


Figure 4.

Panel A

Age<50 at diagnosis



Panel B

Age 50-79 at diagnosis